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# COMPARISON OF CORN LEAF NITROGEN CONCENTRATION AND CHLOROPHYLL METER READINGS

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***ABSTRACT***: Tissue testing of corn leaves for nitrogen (N) concentration is not widely used because it is easier and perhaps more economical to apply sufficient fertilizer than to risk a yield reduction because of an N deficiency. Environmental concerns related to N fertilizer will require producers to improve N management practices to reduce the potential for nitrate leaching. Applying fertilizer N on an "As Needed" basis rather than using a "Lump Sum" approach has both environmental and economic advantages. Corn leaf disk N concentrations and SPAD 502 chlorophyll meter readings from N rate studies were compared at silking for a variety of hybrids at several locations. Data indicated that chlorophyll meter readings correlated well with leaf N concentrations for a given hybrid and location. Calibration of chlorophyll meters to determine crop N status may not be practical because of the unique "greenness" characteristics of different hybrids. However, normalization procedures can be used to standardize the tissue testing approach across hybrids, locations, and growth stages. This can be achieved by comparing chlorophyll meter readings from well fertilized rows to those from the test area. Application of this technology by consultants and producers may require an adequately fertilized area of the field that can be used as a reference for local growing conditions.

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## INTRODUCTION

Testing of corn leaf tissue to compare leaf N concentrations with critical levels is a well established procedure to document a crop N deficiency. Established critical levels in the ear leaf of corn at anthesis are in the range of 27.5 to 28.0 mg N/g (1,2). Comparable values for leaf disk N concentrations are in the range of 35.5 to 36mg N/g (3). In the past, this technology has typically been used as a diagnostic tool rather than a management tool to improve fertilizer N utilization by corn.

Past research indicates a strong positive correlation between leaf N concentration and leaf chlorophyll content (3,4,5). This relationship should make it possible to use leaf chlorophyll content to estimate crop N status and thereby determine the need for additional N fertilizer. Any additional N fertilizer could be applied in irrigation water or by spoke injectors under rainfed conditions. However, Sanchez et al. (6) indicated that chlorophyll content can be affected by water stress, and thus make calibration difficult. Therefore, use of chlorophyll content as an N management tool could be confounded by crop water status. Application of this approach to N management would require that procedures be developed to rapidly and accurately determine leaf chlorophyll content so that corrective measures could be taken if necessary. The recent introduction of a commercially available hand-held chlorophyll meter makes N management based on leaf chlorophyll content a reality.

The objectives of this research were to quantify the relationship between leaf N concentration and the SPAD 502 chlorophyll meter readings (Minolta Corp, Ramsey, NJ)[1] for corn production. Specific goals were to evaluate the potential for calibration of chlorophyll meters across growth stages and plant cultivars for the purposes of evaluating crop N status and scheduling fertigation.

## MATERIALS AND METHODS

Four existing repticated N rate studies in Nebraska under irrigation and one N rate study in Illinois under rainfed conditions were used to compare leaf disk N concentrations and SPAD 502 chlorophyll meter readings of corn. The objectives of each study were different, and therefore, the experimental design used at the various locations was different. Specifics of each experiment are noted in Table 1.

1. Mention of trade names and sources of equipment are not an endorsement of the product but are for convenience of the reader.

**Table 1.** Characteristics of studies used to compare leaf N concentration and chlorophyll meter readings.

| Location | N Rates (kg/ha) | Hybrids* | N Sources | Growth Stages** |
|---|---|---|---|---|
| Urbana, IL | 0, 67, 134, 202, 269 | 873xLH51, LHE136xLH62 | Calcium nitrate<br>Urea<br>Urea + Nitrification Inhibitor | R1 |
| Page, NE | 0, 90, 179, 269, 90+90SD, 179SD | P3362, P3417 P3467, P3503 PX9513, PX9524 | Ammonium nitrate | R1 |
| Schuyler, NE | 0, 90, 179, 269 | P3162, P3379, P3180, P3362, P3417, P3467 | Ammonium nitrate | V8 -R3 |
| Shelton, NE | 0, 67, 202 | P3162, P3362, P3379, P3503, N1-N6 | Anhydrous ammonia | R1 |
| Shelton, NE | 0, 75, 150, 225, 300 | P3379 | Ammonium sulfate | R1 |

*Cultivar numbers preceded by P = Piononer hybrids and N = proprietary hybrids.
**Growth stages designated according to Hanway (7).



One leaf disk (1-cm diameter) was collected from the uppermost expanded leaf before silking or from the ear leaf after silking from each of approximately 60 randomly selected plants. A specially fabricated leaf punch was used to collect the leaf disks (Precision Machine Co., Lincoln, NE 68503). Individual disks were taken midway between the sheath and leaf tip and midway between the midrib and edge of the leaf. These sampling guidelines where chosen to minimize data variability among leaves between plants and positions within a leaf. Concurrently, consideration was given to developing a simple and reliable plant sampling strategy (data not shown). Leaf disks were dried, counted, and weighed prior to compositing for Kjeldahl digestion of the intact disks. Nitrogen concentrations were determined by automated wet chemical procedures (8). Similarly, chlorophyll meter readings were taken from the same relative plant leaf and position on the leaf from 30 randomly selected plants. Chlorophyll meter readings from each plot were averaged for comparison with leaf N concentrations.

Data from selected studies were plotted by N rate to illustrate the effects of crop growth stages, cultivars, fertilizer N sources, and timing of fertilizer application on calibration of the SPAD 502 chlorophyll meters.

## RESULTS AND DISCUSSION

Leaf greenness quantified by the SPAD 502 chlorophyll meter represents a unitless relative measurement of leaf chlorophyll content because the mass of the sample is not determined. It is theoretically possible to convert the meter readings to a measure of specific chlorophyll content (reading area) because the optics of the meter are based on a 2x3 mm sensor. However, traditional wet chemical procedures used to determine leaf chlorophyll content are usually based on the mass of the tissue. Therefore, data expressed herein are given as direct chlorophyll readings because the relationship between meter readings and chlorophyll content have previously been established (9).

Calibration of the SPAD 502 chlorophyll meter for the purposes of estimating leaf N concentration was separated into several components that were considered to affect the analytical relationship and that could influence application of the technology. In general, corn leaf greenness is expected to increase as soil N availability increases. However, genetic differences between cultivars are also recognized to affect greenness. Other factors that could influence calibration of the SPAD 502 meters include stage of crop growth and possibly the time and form of N application.

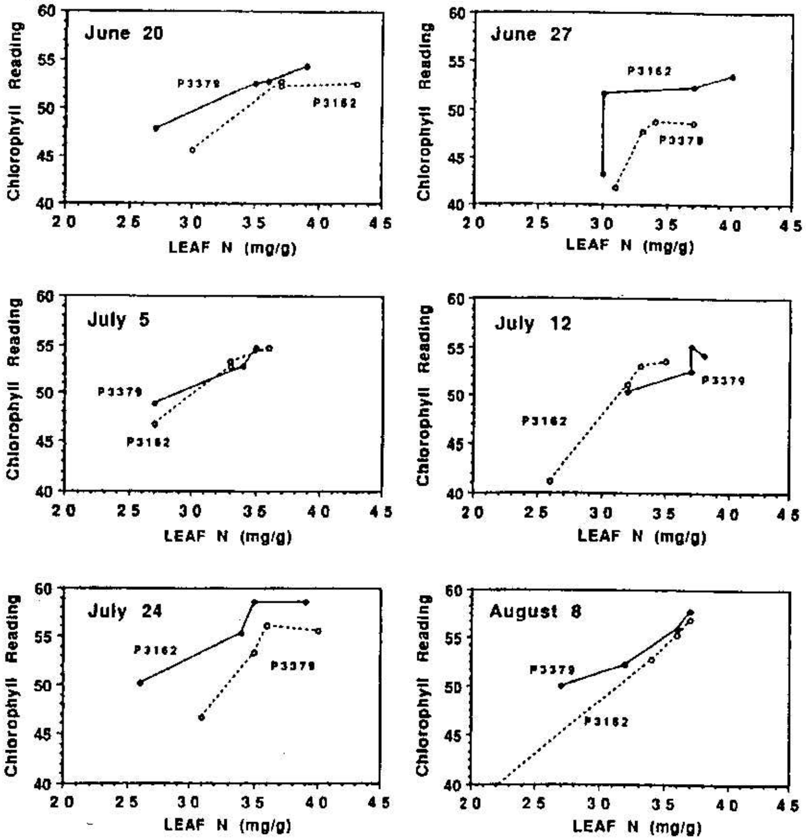


Figure 1. Comparison of leaf disk N concentrations and chlorophyll meter readings of irrigated corn at six sampling dates for two hybrids.

*Effect of Crop Growth Stage:* Calibration of the chlorophyll meters throughout the growing season presents a unique challenge in that a sampling strategy must be developed that is indicative of soil N availability, regardless of crop growth stage. For the past several decades, the ear leaf or an adjacent leaf have traditionally been analyzed for Kjeldahl N concentration to evaluate crop N status after silking (1,2). Prior to silking, the uppermost expanded leaf (*i.e.*



youngest leaf with a fully exposed collar) was used for calibration because younger leaves showed considerably greater variation in chlorophyll meter readings along the length of the leaf (data not shown). Younger leaves generally tended to have lower chlorophyll meter readings. Further, areas near the base of younger corn leaves usually have significantly lower readings than near the leaf tip until after the leaf collar is fully exposed. Once the leaf collar is exposed, variation in meter readings along the length of the leaf was minimized. Meter readings taken near the midrib tended to be slightiy higher than near the leaf margin. This difference is attributed to a thicker leaf near the midrib than near the margin (data not shown).

Data from the Schuyler, Nebraska study for two selected hybrids show that meter calibration curves varied with the stage of plant growth (Fig. 1) in that the shape of the relationship changes with time. Further, the shapes of the calibration curves were different for each hybrid. The gradual decline in leaf N concentration for all fertilizer N rates until near silking (~July 20) was expected because of the rapid growth before silking. After silking, leaf N concentrations continued to decline at the low fertilizer N rates, but remained nearly constant at the the higher N rates.

Greater significance should be placed on the shape of the calibration curve with time than the absolute value of the meter readings because there are currently no procedures to standardize readings between dates. This fact may represent a limitation in the application of chlorophyll meter data even though it is possible to adjust the meter readings to compensate for differences between meters. Although comparison of chlorophyll meter readings between dates may be questionable, data collected on a given date should provide a relative comparison of crop response to fertilizer N rates. For example, chlorophyll meter readings at the 90 kg/ha N rate for both hybrids were similar to higher N rates early in the season (Fig. 2). As the growing season progressed, meter readings for both hybrids showed the development of an apparent N stress at the 90 kg/ha N rate. Late in the growing season, care must be taken not to confuse lower chlorophyll meter readings with a natural yellowing of the leaves associated with senescence. Again, a relative comparison of chlorophyll meter readings across N rates on a given date should provide a reasonable index of crop N status aside from any minor changes in maturiry induced by N rates.

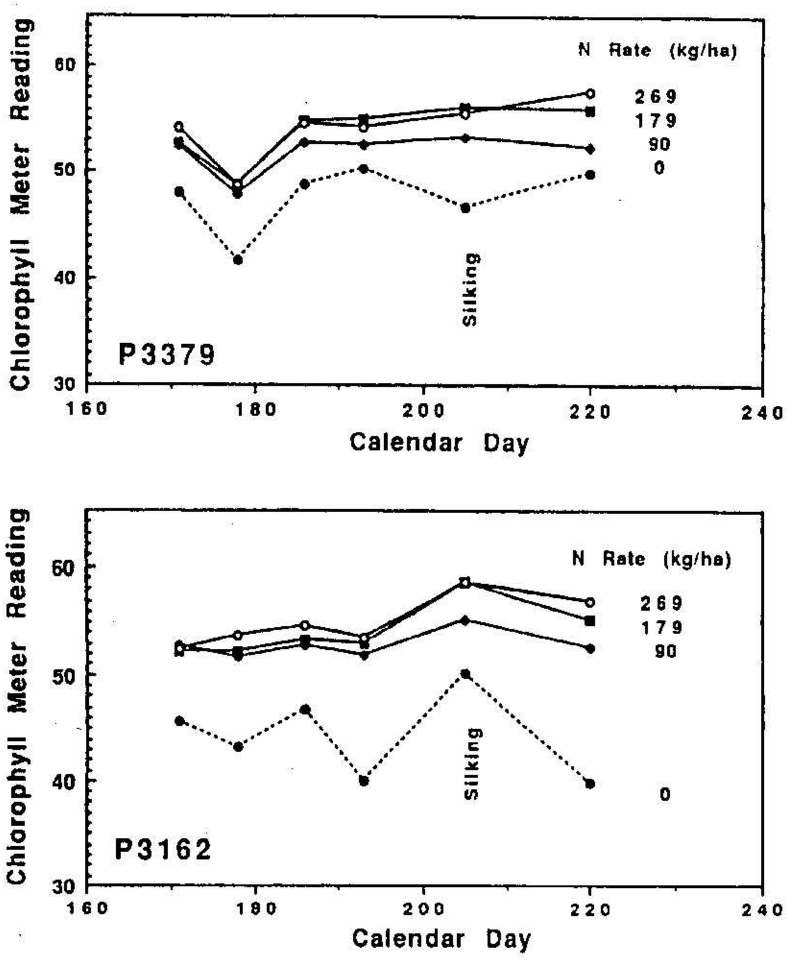


Figure 2. Seasonal variation in chlorophyll meter readings for two maize hybrids at four fertilizer N rates.



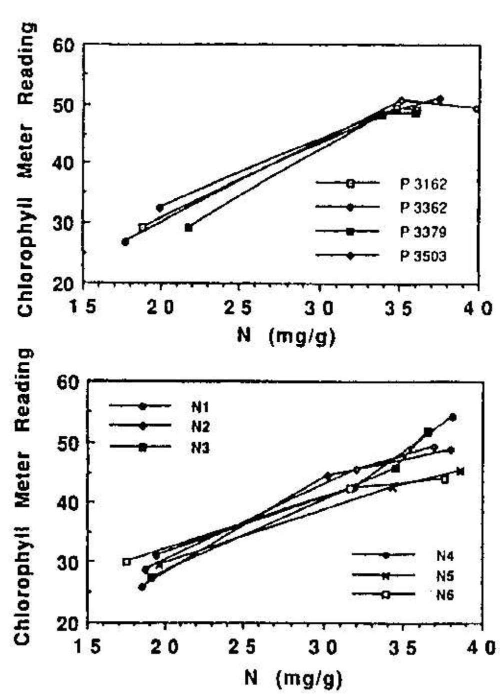


Figure 3. Comparison of leaf disk N concentrations and chlorophyll meter readings of ten irrigated corn hybrids grown at three fertilizer N rates.

*Cultivar Effects:* The large genetic diversity in the study at Shelton, Nebraska showed that all ten hybrids generally followed similar trends in terms of leaf disk N concentration and chlorophyll meter readings (Fig. 3). Significant differences in both chlorophyll meter readings and leaf N concentration existed across the three N rates (Table 1).

Some hybrids reached near maximum leaf N concentrations and chlorophyll meter readings at the 67 kg/ha N rate, while others required nearly 202 kg N/ha to attain maximum chlorophyll meter readings.

The general linearity of the relationship between leaf disk N concentrations and chlorophyll meter readings is encouraging, but specific trends at high fertilizer N rates demonstrate unique differences between hybrids. For example, Pioneer

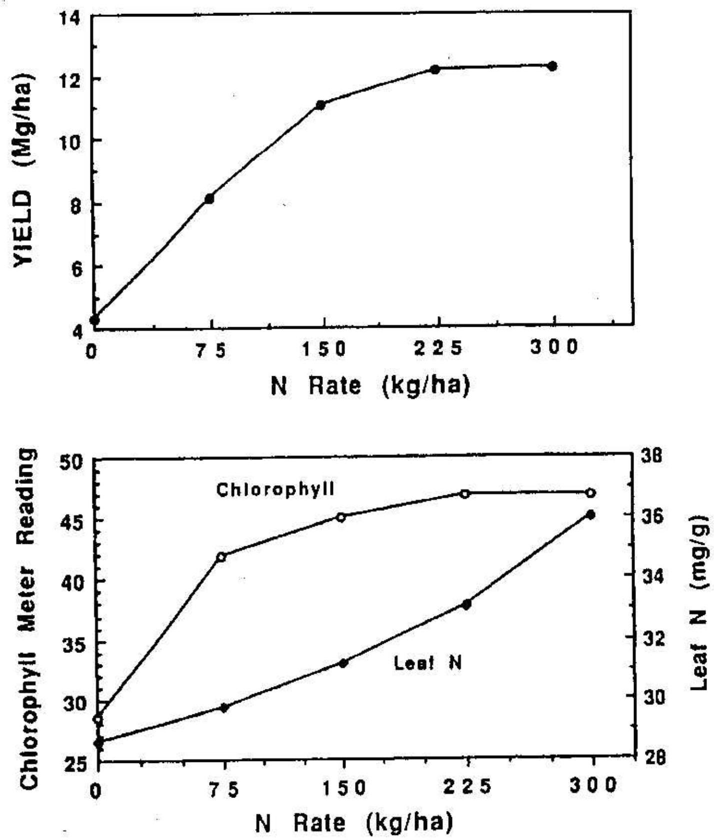


**Figure 4.** Effect of N rate on irrigated maize yield and chlorophyll meter readings and leaf disk N concentrations at anthesis.

brand hybrid 3503 was able to accumulate higher than average leaf N concentrations at silking, but with no notable increase in chlorophyll meter readings. In contrast, the hybrid noted as N1 continued to accumulate leaf N and increase chlorophyll meter readings up to the 202 kg/ha N rate. This later hybrid was also notably greener than the others throughout the growing season. It is not obvious from this study how excessively high fertilizer N rates would affect either



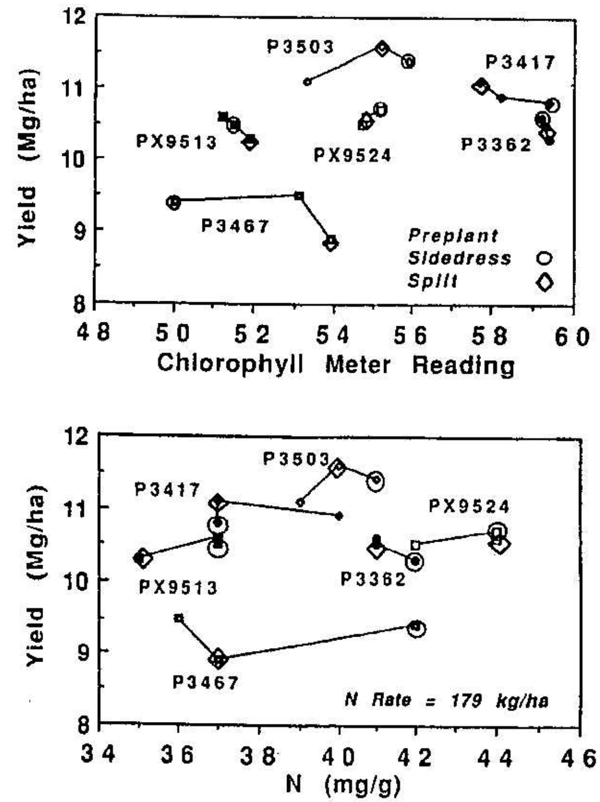


**Figure 5.** Effect of N fertilizer timing on leaf disk N concentrations, chlorophyll meter readings, and maize yields.

leaf N concentrations or chlorophyll meter readings. However, there is some indication that as fertilizer N rates increase, chlorophyll meter readings tend to approach a plateau and that leaf N concentrations no longer reflect the increase in N availability.

***Effect of Overfertilizarion:*** Excessively high rates of N fertilizer application can reduce profitability and threaten ground water quality. However, it is economically difficult to justify limiting N application to grain crops to the extent

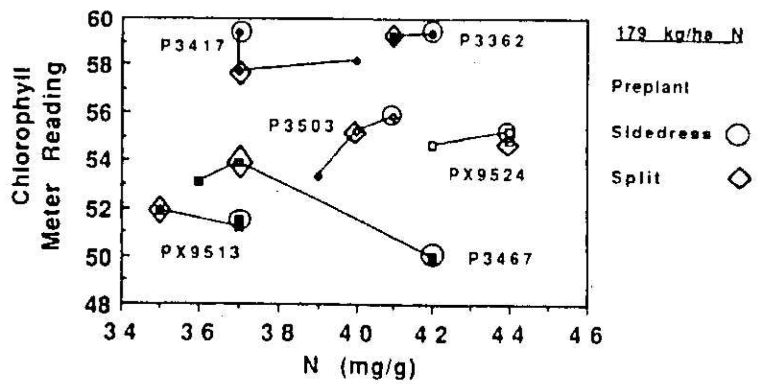


**Figure 6.** Comparison of leaf disk N concentrations and chlorophyll meter readings for irrigated maize fertilized three ways.

of risking an N deficiency which is likely to result in a yield reduction. This profitability relationship is because of the relatively inexpensive nature of N fertilizer in the U.S. and the expectation of higher yields with increasing rates of N fertilizer application.

Only the study sponsored in part by the Tennessee Valley Authority at Shelton, Nebraska included a sufficiently wide range of N application rates that demonstrated a response to N fertilizer that could be used to evaluate calibration of the meter at fertilizer N rates higher than necessary to attain maximum yields. Both grain yield and chlorophyll meter readings reached a plateau at N rates beyond the 225 kg/ha N rate (Fig. 4). In contrast, leaf disk N concentrations continued to increase to the 300 kg/ha N rate. In this case, the crop was not able to capitalize on the additional N uptake and did not produce additional grain above the 225 kg/ha N-rate.

The literature generally suggests a positive correlation between crop N uptake, leaf N concentration, leaf chlorophyll content, and grain yield. These data support the above observations, but further suggest that ear leaf chlorophyll content at anthesis reaches a plateau at high N rates (Fig.4). This trend was similar to the yield response to N fertilizer and indicates that leaf chlorophyll content or leaf greenness, as measured with the SPAD 502 chlorophyll meter, may provide a better estimate of potential yield than does leaf N concentration.



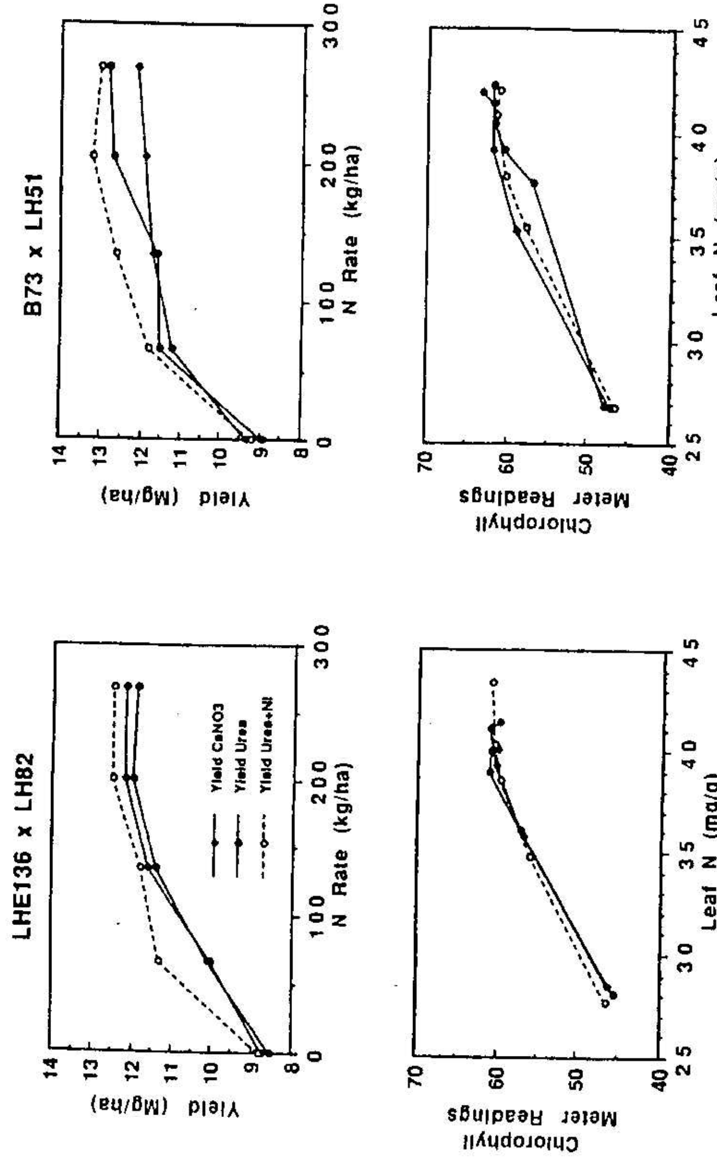


**Figure 7.** Effect of fertilizer N rate and source on maize yield and leaf disk N concentrations and chlorophyll meter readings at anthesis.

***Effect of Fertilizer Timing:*** Practical application of tissue testing to evaluate crop N status necessitates a universal strategy for data interpretation, regardless of when fertilizer is applied. Data from the Page, Nebraska, study showed that the timing of N fertilizer application confounded calibration of the SPAD 502 chlorophyll meter and made interpretation of the field data more difficult. A total of 179 kg N/ha was applied to the six hybrids either at i) planting time, ii) evenly split between planting time and sidedress at the V8 growth stage, or iii) at sidedress time. No consistent trend in leaf disk N concentrations or chlorophyll meter readings at silking or grain yields at harvest was observed for the three N application strategies across the various hybrids, although yields across hybrids were different (Fig. 5). Only Pioneer brand hybrid 3467 had significantly different leaf N concentrations and chlorophyll meter readings because of the time of N application.

The different relationships between leaf N concentration, chlorophyll meter readings, and yield in response to time of N application between the six hybrids are attributed to genetic factors that control the various metabolic processes. This study illustrates that caution should be taken when calibrating the SPAD 502 chlorophyll meter against leaf N concentration, even within a maize hybrid, because of apparent metabolic differences induced by time of N application (Fig 6). The significance of differences in leaf N concentrations or chlorophyll meter readings for Pioneer brand hybrid 3467 is unknown because time of N application had no significant affect on yield within a hybrid.

***Effect of N Source:*** Various sources of N fertilizer are expected to have similar effects on chlorophyll meter readings and leaf N concentrations as does timing of N application. This is because N transformations in soil affect crop N availability and crop N uptake. The crop, on the other hand, may have a preference for nitrate versus annmonium forms of N. These effects are illustrated by the Illinois study where the urea plus nitrification inhibitor form of fertilizer consistently produced the highest yields (Fig. 7). However, neither chlorophyll meter readings nor leaf N concentrations showed no consistent difference between the N sources. Apparently those factors contributing to the higher yield where the nitrification inhibitor was used were expressed before or after the time of tissue sampling.



## CONCLUSIONS

Calibration of the SPAD 502 chlorophyll meter against leaf N concentration in a general sense is possible because of the close relationship between leaf N concentration and leaf greenness. However, factors such as crop growth stage, hybrid, timing of N fertilizer application, and N source limit the feasibility of using the SPAD 502 chlorophyll meter as a substitute for determining leaf N concentration. Although critical levels have been established for corn leaf N concentrations, the limited data contained in this report indicates that chlorophyll meter readings at anthesis were equally well or better correlated with yields than were leaf N concentrations. Because chlorophyll meters provide a unitless indication of leaf greenness, utilization of this technology will require normalizing the data relative to an adequately fertilized area of the field. It should be noted that normalized calibration relationships will maintain the same general form as the onginal data, but will utilize a common range of values. In this way, normalized data are essentially internally calibrated for each field, hybrid, stage of growth, and set of cultural practices. Normalized data lends themselves to a common strategy for interpretation.

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